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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/990,705	11/21/2001	Judith K. Gwathmey	JGT-004	3899

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EXAMINER	
AFREMOVA, VERA	

ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 01/13/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/990,705	GWATHMEY ET AL.	
	Examiner	Art Unit	
	Vera Afremova	1651	

-- The MAILING DATE of this communication appears on the c ver sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *29 October 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disp sition of Claims

4)☒ Claim(s) *1-26* is/are pending in the application.

4a) Of the above claim(s) *14-26* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

In view of the appeal brief filed on 10/29/2004, PROSECUTION IS HEREBY REOPENED. New grounds of rejection are set forth below.

To avoid abandonment of the application, appellant must exercise one of the following two options:

(1) file a reply under 37 CFR 1.111 (if this Office action is non-final) or a reply under 37 CFR 1.113 (if this Office action is final); or,

(2) request reinstatement of the appeal.

If reinstatement of the appeal is requested, such request must be accompanied by a supplemental appeal brief, but no new amendments, affidavits (37 CFR 1.130, 1.131 or 1.132) or other evidence are permitted. See 37 CFR 1.193(b)(2).

Status of claims

Pending claims 1-13 (amendment filed 11/18/2003 and re-submitted same on 1/30/2004) are under examination in the instant office action.

Claims 14-26 were withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to nonelected inventions without traverse. A complete reply to the final rejection must include cancellation of nonelected claims or other appropriate action (37 CFR 1.144) See MPEP § 821.01.

Claim Rejections - 35 USC § 112

New matter

Claims 1-13 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not

described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Insertion of the limitations drawn to successively exposing tissues to amounts of calcium "**decreasing from about 1-2 μM**" and than "**increasing from about 1-2 μM**" has no support in the as-filed specification. The insertion of this limitation is a new concept because it neither has literal support in the as-filed specification by way of generic disclosure, nor are there specific examples of the newly limited genera that would show possession of the concept of the step-wise successive exposure of tissues to calcium amounts "decreasing from about 1-2 μM" and than step-wise successive exposure of the dissociated tissues to calcium amounts "increasing from about 1-2 μM".

There are two embodiments in the disclosure that relates and describes calcium amounts in the method for isolating cardiac cells.

First embodiment describes generic concept of exposing tissue to a solution with decreasing amounts of calcium, enzymatically-digesting tissue and than exposing the digested tissue to increasing amounts of calcium. See page 5, lines 10-20. This generic embodiment does not indicate what particular amounts calcium are intended. This embodiment does not indicate starting, intermediate and/or final concentrations of calcium. The particular example (example 1, page 12) that relates to this first generic embodiment demonstrates a single step decrease in calcium concentration from 1mM to 0 mM (page 12, lines 25-26) and than successive exposure to solutions with increasing concentrations of calcium 0 mM/100 μM/250μM /250μM/500μM (page 12, lines 35-37). The disclosed calcium concentrations are different from that they are

claimed. The step of decreasing calcium amounts as described is not a successive step-wise decrease of calcium amounts as claimed but one step decrease. The described single step decrease of calcium concentration is not a step-wise calcium decrease by 1-2 μM each consecutive step as argued in the Appeal Brief (page 5, par. 5, line 4).

Second embodiment describes repetitive use of solutions with the same concentration of calcium 1-2 μM (see page 6, line 16 and line 23). The particular example that relates to this embodiment demonstrates repetitive use of the solution containing 1-2 μM calcium (page 14, line 26-27). Than cells are exposed to enzymatic and rinsing solution containing constant amount 30 μM calcium chloride (page 14, line 30 and page 15, line 1). Thus, the disclosed step of decreasing calcium amounts is not a successive step-wise decrease of calcium amounts as claimed. The described method comprises using the same low calcium solution. The described method does not comprise a step-wise calcium decrease by 1-2 μM each consecutive step as argued in the Appeal Brief (page 5, par. 5, line 4) and as encompassed by the claims. Further, the disclosed calcium concentrations in increasing step are different from that they are claimed. The described step of increasing calcium is not a step-wise calcium increase by 1-2 μM each consecutive step as argued in the Appeal Brief (page 5, par. 5, line 4) and as encompassed by the claimed invention.

Thus, the original as-filed specification contains disclosure about generic "decreasing" and "increasing" calcium amount in the method for isolating cardiac cells. However, there is no disclosure about step-wise successive exposure of tissues to calcium amounts "decreasing from about 1-2 μM" and than step-wise successive exposure of dissociated tissues to calcium amounts

"increasing from about 1-2 μM". There is no sufficient support for limitations "decreasing from about 1-2 μM" and "increasing from about 1-2 μM" as presently claimed.

This is a matter of written description, not a question of what one of skill in the art would or would not have known. The material within the four corners of the as-filed specification must lead to the generic concept. If it does not, the material is new matter. Declarations and new references cannot demonstrate the possession of a concept after the fact. Thus, the insertion of limitations "**decreasing from about 1-2 μM**" and "**increasing from about 1-2 μM**" is considered to be the insertion of new matter for the above reasons.

Claim Rejections - 35 USC § 112

Claims 1-13 remain rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 and 13 remain indefinite and unclear with regard to concentration or amounts of calcium chloride. The presently claimed phrases "decreasing from about 1-2 μM" and "increasing from about 1-2 μM" do not make logical sense in the claimed method. If concentration falls from A to B, it will start to raise from B to A. Therefore, it cannot start to raise from A. The claims are rendered indefinite by a combination of the phrases "decreasing" and "increasing" starting with the identical amounts 1-2 μM for both "decreasing" and "increasing" steps. On one hand, the claimed method requires to decrease calcium amounts for cell dissociation/isolation and to increase calcium amounts for restoration of cellular function of the isolated cardiomyocytes. However, the amounts of calcium as claimed are identical. Thus,

the concept of manipulating calcium amounts, if there are any intended changes, is unclear as claimed.

Claims 6 and 10 appear to re-state the same limitations drawn to calcium decrease and increase that are recited in claim 1. Thus, claims 6 and 10 are indefinite and/or fail to further limit.

With respect to claims 12 and 13 it is noted that the claimed amount of 8.8 mg of ascorbic acid fails to indicate for what volume of medium it is intended. It is suggested to insert ---8.8 mg/500 ml--- as disclosed on page 11, line 12 and line 19.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tytgat (IDS reference; Cardiovascular Research. 1994, 28: 280-283) and Kruppenbacher et al. (IDS reference; Naturwissenschaften. 1993, 80: 132-134) taken with the ATCC catalogue and Kang et al. (IDS reference; Proc. Natl. Acad. Sci. USA. 1994. 91: 9886-9890).

Claims are directed a method of isolating adult cardiac cells comprising step of obtaining a tissue sample from a subject, step of successively exposing the tissue to a first solution with decreasing amounts of $CaCl_2$, wherein the fist solution comprises NaCl, HEPES, $MgCl_2$, KCl and sugar and has pH about 7.4; step of disassociating the tissue with an enzyme solution; step of

repeatedly resuspending the disassociated tissue into second solution with increasing amounts of $CaCl_2$, wherein the second solution comprises L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES and antibiotic and has pH 7.4 in order to obtain the isolated cells. The amounts of calcium chloride during at least some period of decreasing and increasing steps are intended to be about 1-2 μM.

Some claims are further drawn to incubating isolated cells in a mixture of carbon dioxide and air at temperature 37 degree C. Some claims are further to exposing the tissue to a first solution at 37 degree C at 4 ml/min for 3 minutes. Some claims are further drawn to the use of digestive enzyme protease or collagenase in the enzyme solution a method of isolating cells. Some claims are/are further drawn to particular concentration of the ingredients in the first and in the second solutions.

The reference by Tytgat teaches method of isolating cardiac cells by enzymatic digestion wherein method comprises step of exposing cardiac tissue to normal Tyrode solution (1.8 mM $CaCl_2$), then step of exposing tissue to calcium-free Tyrode solution (0 mM $CaCl_2$) in order to cause cessation of heartbeat $CaCl_2$, then step of enzymatically digesting cardiac cells with collagenase and protease, then step of exposing digested cells to "low calcium" solution (0.18 mM $CaCl_2$) and further exposing digested cells to normal Tyrode solution (1.8 mM $CaCl_2$). See page 280, col. 2 to page 281 col. 1.

Thus, the cited reference by Tytgat clearly teaches concept of decreasing calcium amounts in order to cause cessation of heartbeat, enzymatically digesting cardiac tissue cells and then increasing calcium amounts for gradual recalcification of the digested cardiac cells in order to restore cardiac cell function. The amounts of $CaCl_2$ fall from 1.8 mM to zero and raise from

zero to 0.18 mM/1.8 mM. Thus, the amounts of calcium chloride during at least some period of the decreasing and increasing steps are about 1-2 μM $CaCl_2$.

The solutions employed in the method of Tytgat comprise NaCl, HEPES, $MgCl_2$, KCl and glucose at identical or substantially similar concentrations as required by claimed method for the first solution and for the enzyme solution such as 137 mM NaCl, 11.6 mM HEPES, 0.5 mM $MgCl_2$, 5.4 mM KCl and 5 mM glucose (see table at page 280). The enzymes in the method of Tytgat are identical to enzymes in the claimed method. The solutions have pH about 7.4. The cited method of Tytgat encompasses the use of the same culture conditions such as mixture of carbon dioxide and air and temperature 37 degree C as required by the claimed method.

The method of Tytgat is different from the claimed invention by some of the ingredients in the second or final solution(s) in the method for isolating cardiac cells. The final solutions employed in the method of Tytgat are lacking L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic and antibiotics.

However, it is known to use these ingredients including L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic and antibiotics in solution(s) in the methods for isolating and culturing cardiac cells.

For example: Kruppenbacher et al. discloses a method of isolating adult cardiomyocytes wherein the method comprises step of obtaining a heart tissue sample from a subject (page 133, col. 1, lines 14-16) in a normal saline solution; step of successively exposing the tissue to a first buffer solution containing low amount of $CaCl_2$ such as 25 μM (page 133, col. 1, line 24) and 110 mM NaCl, 1.2 mM $MgSO_4$, 2.6 mM KCl and 11 mM glucose (page 133, col. 1, lines 20-22); step of disassociating the tissue with an enzyme solution comprising digestive enzymes protease

(trypsin) and collagenase (page 133, col. 1, line 25) as well as other components of the first buffer solution including same amounts NaCl, $MgSO_4$, KCI and glucose (page 133, col. 1, lines 20-22); step of repeatedly resuspending the dissociated tissue and cells into second solution with increasing amounts of $CaCl_2$ such as 0.2/0.2/1.0 mM and finally into the "M 199" culture medium comprising salts, sodium bicarbonate, creatinine, taurine and antibiotics as clearly disclosed (see page 133, col. 2, lines 5-12). The cited reference discloses incubating isolated cells in a mixture of carbon dioxide and air at temperature 37 degree C (col. 3, line 6). The cited reference discloses step of exposing the tissue to a first solution at 37 degree C at 4 ml/min for 20 minutes (page 13, col. 1, line 26). The method by Kruppenbacher encompasses the use of the medium M199. The medium M 199 is known to comprise additional ingredients required by the presently claimed method including ascorbic acid and sodium pentothenate, for example: see the ATCC catalogue at page 522.

In addition, the reference by Kang et al. is relied upon to demonstrate that solutions suitable for isolating, culturing and maintaining function of cardiomyocytes comprise the presently claimed ingredients at identical or substantially similar concentrations such as 140 mM NaCl, 5 mM HEPES, 1 mM $MgCl_2$, 5 mM KCI and 10 mM (page 9887, col. 1, lines 1-2).

Therefore, it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to practice a method of isolating adult cardiac cells by exposing cardiac tissue to decreasing amounts of calcium in order to cause cessation of heartbeat, enzymatically digesting tissue cells and further exposing the digested cells to increasing amounts of calcium in order to restore cellular function as taught by the references by Tytgat and Kruppenbacher et al with a reasonable expectation of success in isolating viable and active cells

from the tissue as adequately demonstrated by Tytgat and Kruppenbacher et al. The cited methods of Tytgat and Kruppenbacher et al. are substantially similar, if not identical, to the presently claimed method as explained above. With regard to claim limitation drawn to the use of particular nutrients at particular amounts, for example: L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic and antibiotics, it is noted that these ingredients have been known and used in regular animal cell culture media including cardiac cell culture media. It is considered to be within the purview of ordinary skill practitioner to adjust intervals of incubation, pH or amounts of some nutrients with regard to a particular cell tissue culture or experimental system. One of skill in the art is would have been motivated to do so for the expected benefits in maximizing effects related to the cell survival, activity and function.

Thus, the claimed invention as a whole was clearly *prima facie* obvious, especially in the absence of evidence to the contrary.

The claimed subject matter fails to patentably distinguish over the state art as represented be the cited references. Therefore, the claims are properly rejected under 35 USC § 103.

Response to Arguments

Applicants' arguments filed 10/29/2004 in the Appeal brief have been considered but are moot in view of the new ground(s) of rejection.

With respect to the claim rejection under 35 U.S.C. 112-2 applicants argue that the calcium amounts are definite because each step calcium are changed by about 1-2 μM (page 4, line 20 and page 5, par. 5, line 4). This argument does not find support in the as-filed original

disclosure. Moreover, claimed invention says "from about " but it does not say " by about " as argued.

Please, note that Appeal brief filed 10/29/2004 is defective because it does not contain items required under new rules 37 CFR 41.37(c) or they are not under proper heading or in proper order (box 1 on PTOL-462 (Rev. 9-04)). Applicants' arguments with respect to pending claims or claim limitations as argued do not found support on specification pages (box 4 on PTOL-462 (Rev. 9-04)).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Vera Afremova whose telephone number is (571) 272-0914. The examiner can normally be reached from Monday to Friday from 9.30 am to 6.00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached at (571) 272-0926.

The fax phone number for the TC 1600 where this application or proceeding is assigned is (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Vera Afremova

AU 1651

January 7, 2005

V. Afremova

VERA AFREMOVA

PATENT EXAMINER

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